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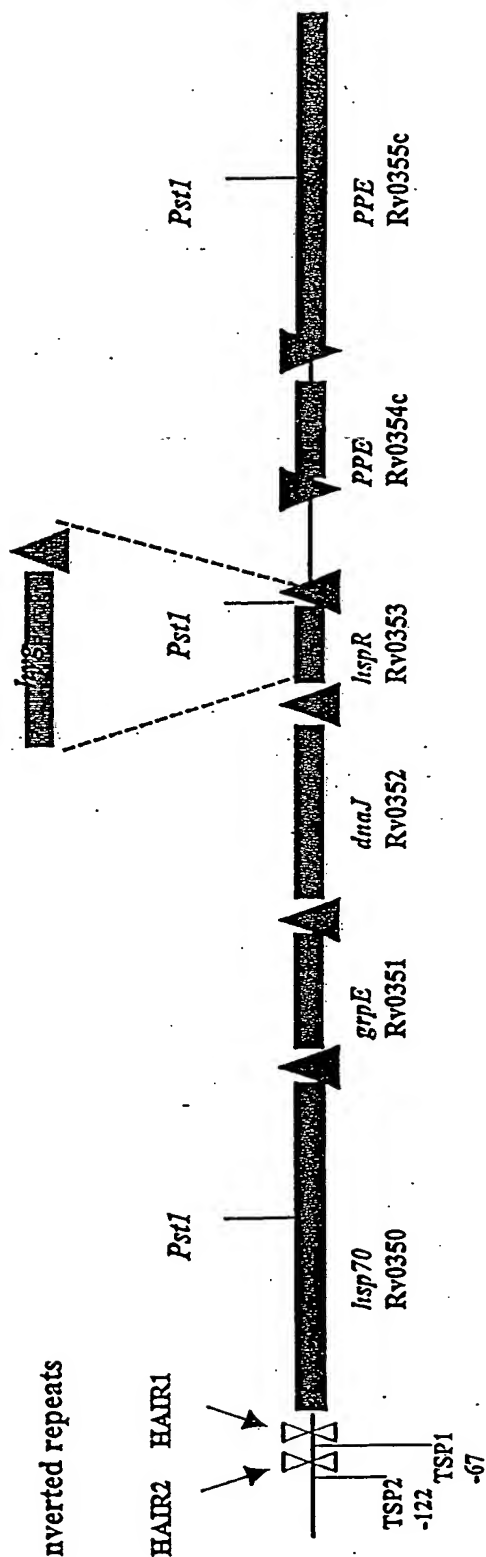
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Figure 1

a Inverted repeats



b

HspR	-	+	+	+	+	+	+
BCG	-	-	+	+	-	-	+
Temp °C	30	30	30	30	48	48	48

c

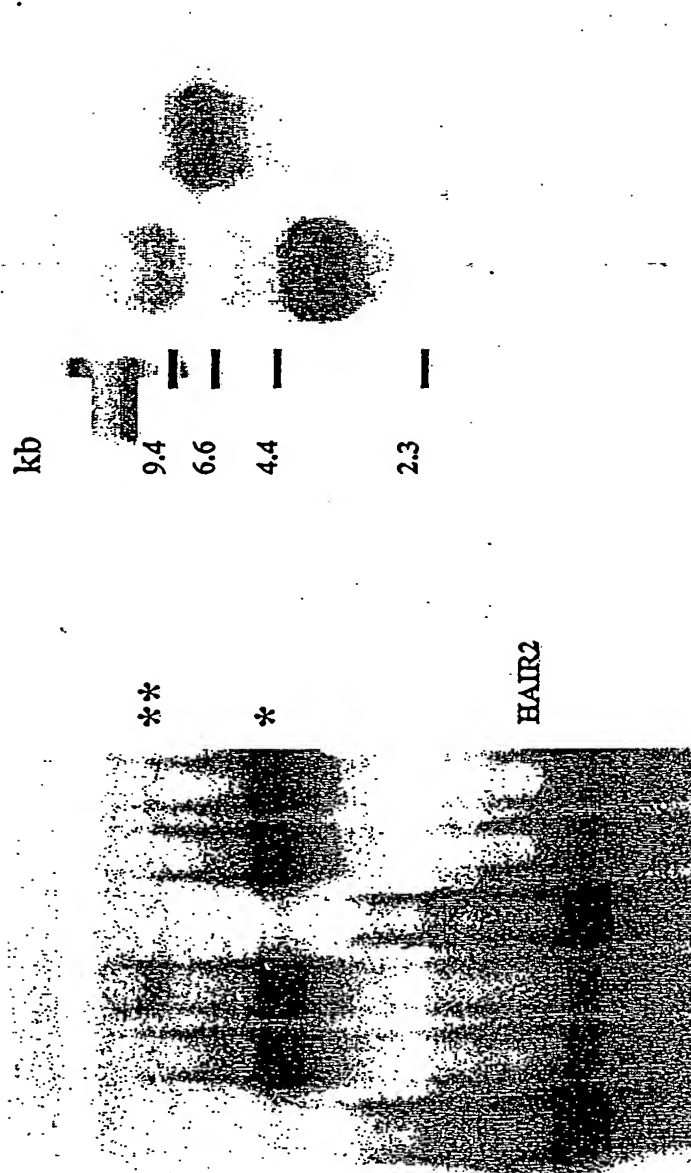
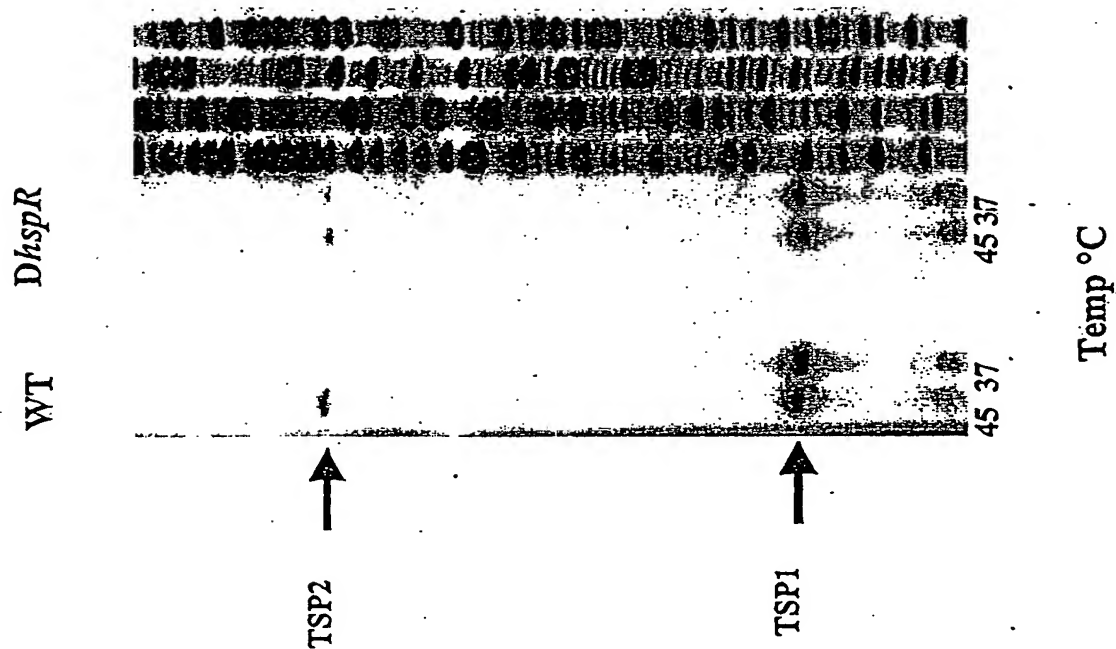


Figure 2

a



b

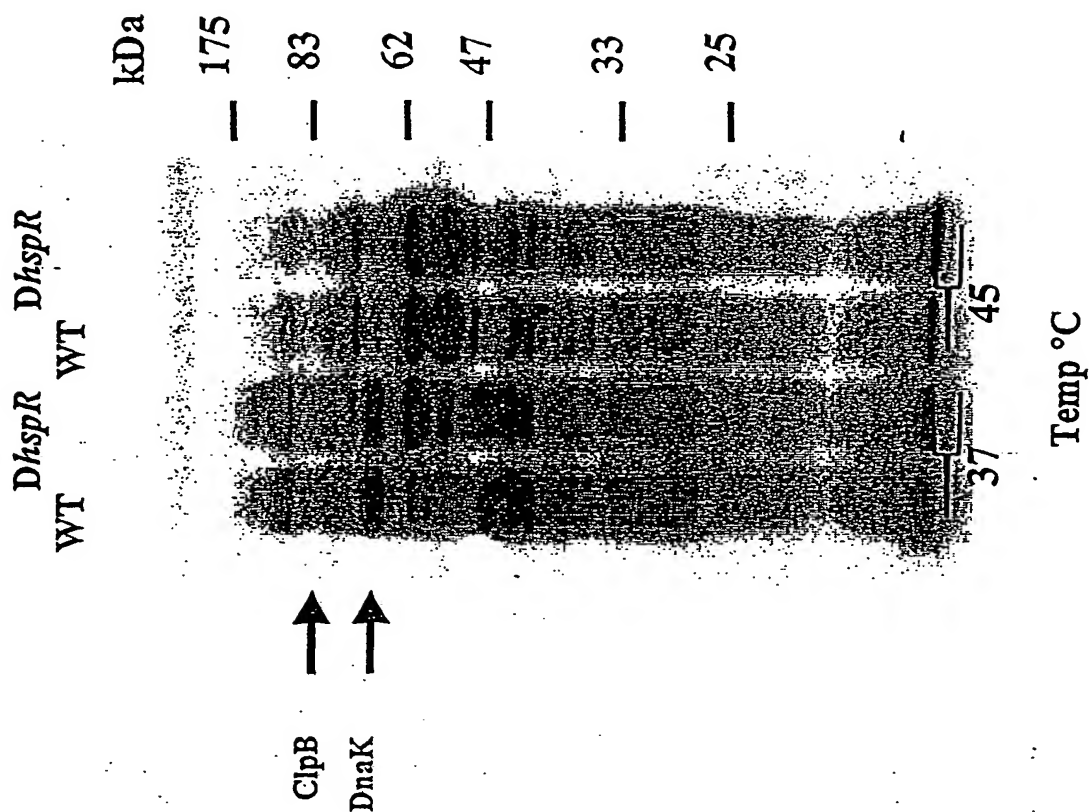


Figure 3

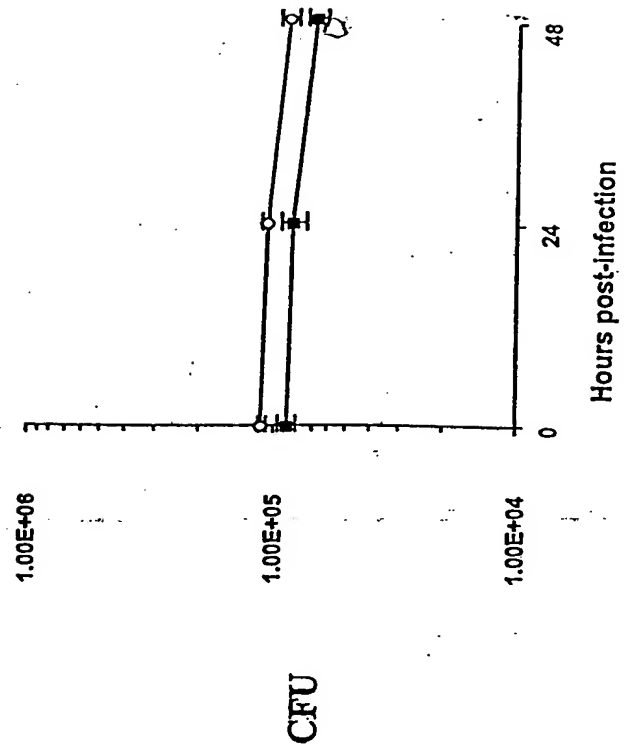
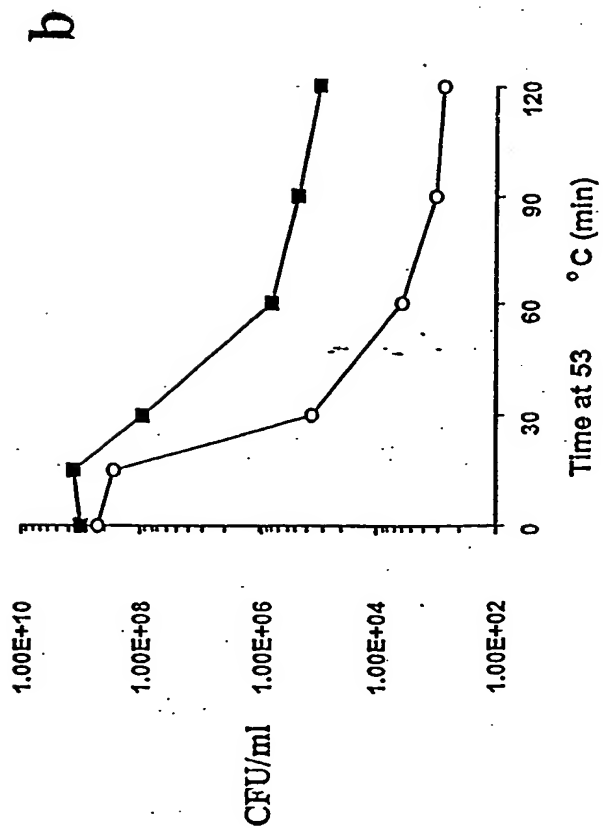
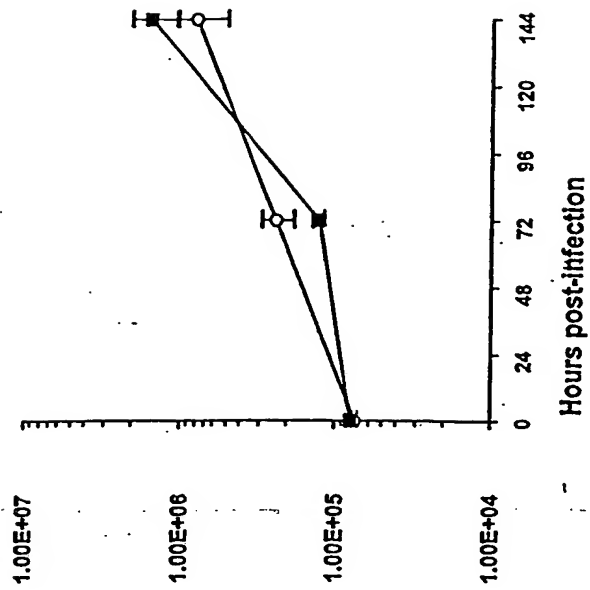
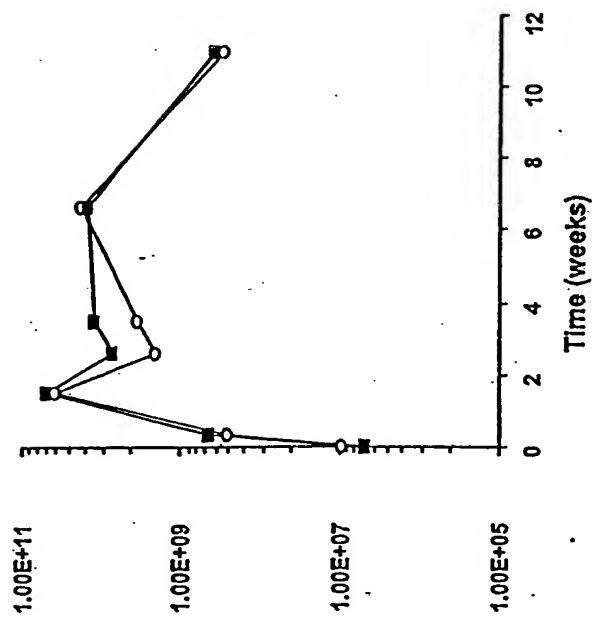


Figure 4

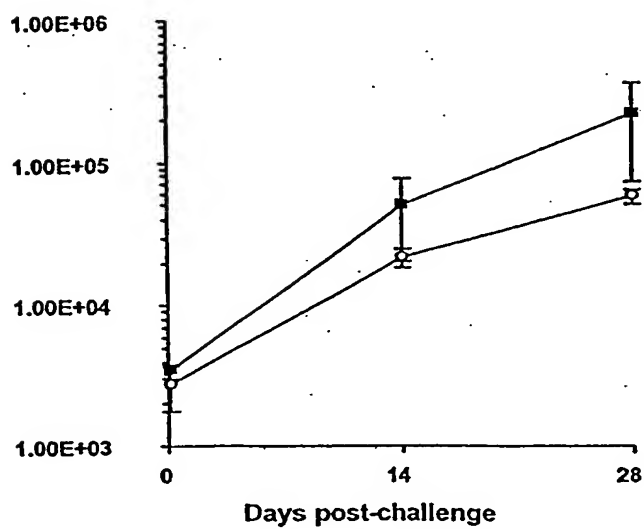
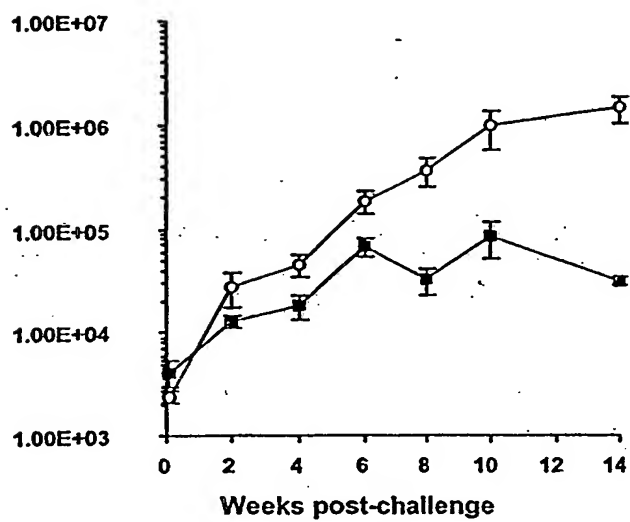
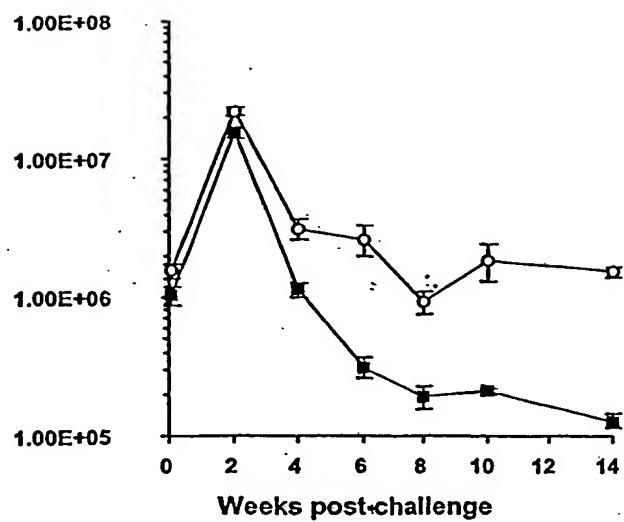
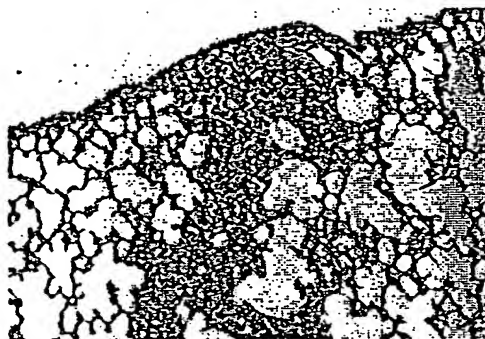


Figure 5

a



b

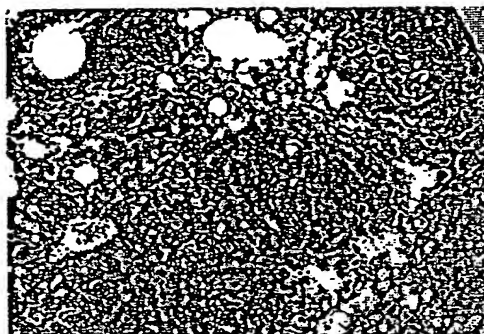


Figure 6

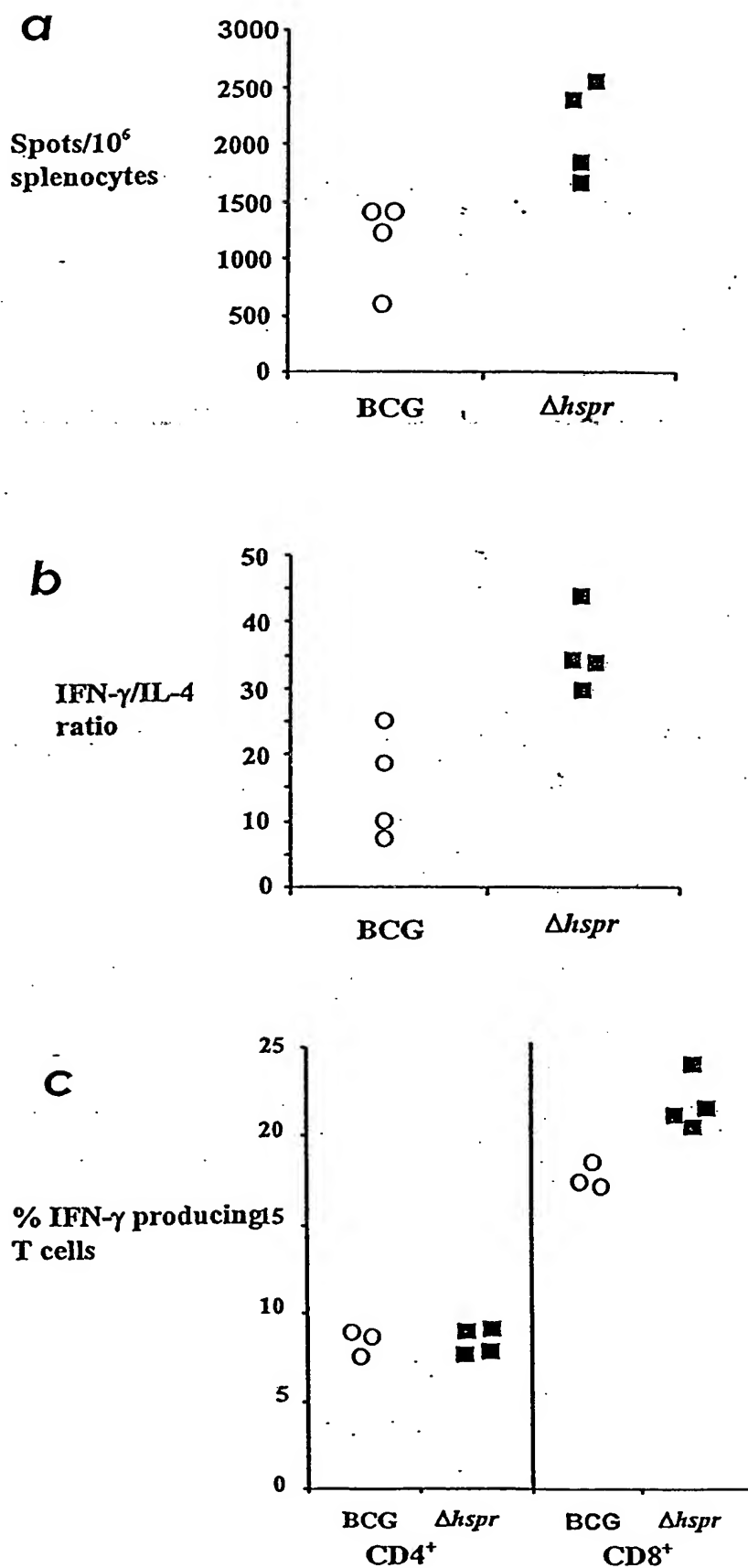
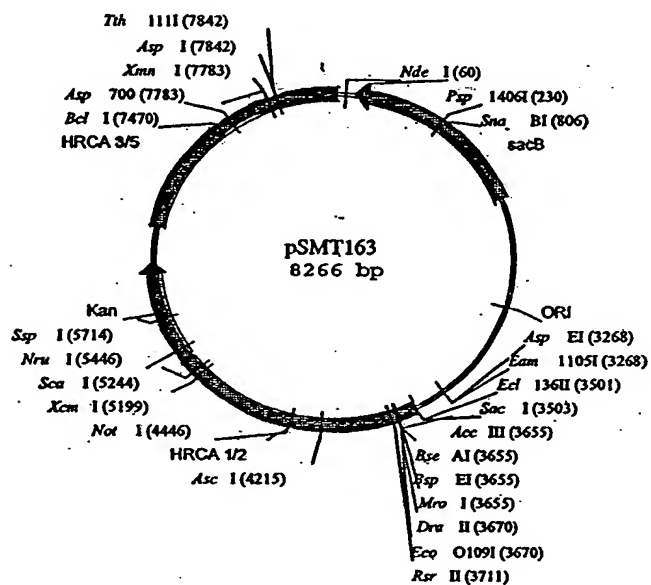


Figure 7. Counterselectable suicide vector for gene replacement of *hrca* in *M.tuberculosis* and *M.bovis* BCG.



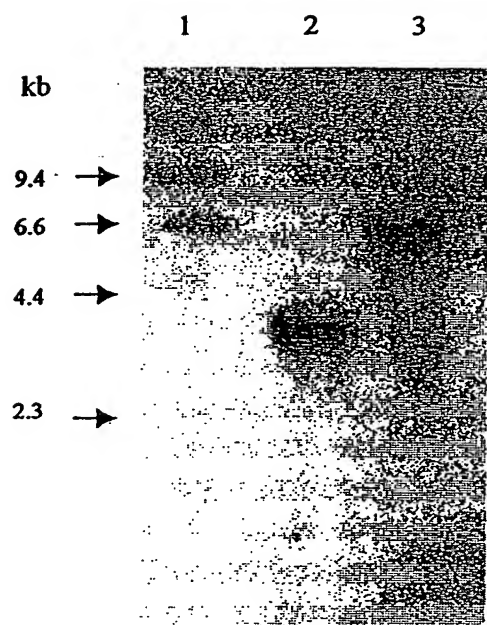


Figure 8. Southern blot of *KpnI* digested gDNA probed with HRCA1/HRCA2. Lane 1, *hindIII* digest of λ DNA; lane 2, *M.tuberculosis* $\Delta hspR$; lane 3, *M.tuberculosis* $\Delta hspR \Delta hrca$.

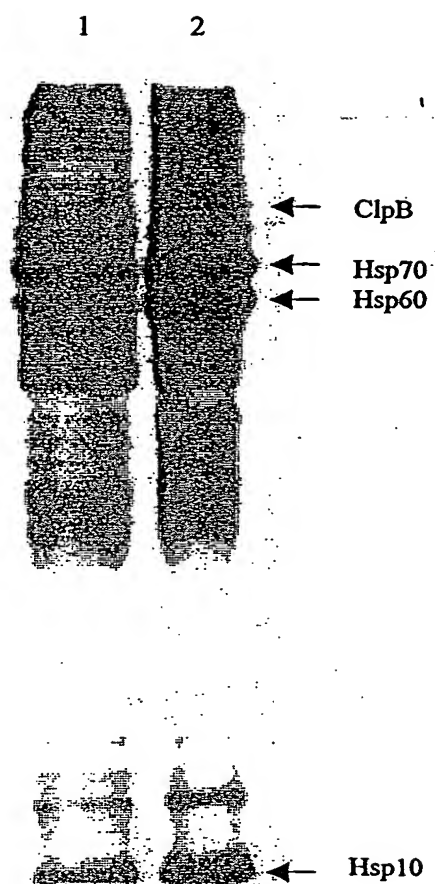


Figure 9. SDS-PAGE showing overexpressed ClpB, Hsp70, Hsp60 and Hsp10 (GroES) in the *hspR* and *hrca* deleted strain. Lane 1, wild type *M. tuberculosis* H37Rv; lane 2, *M. tuberculosis* $\Delta hspR \Delta hrca$.

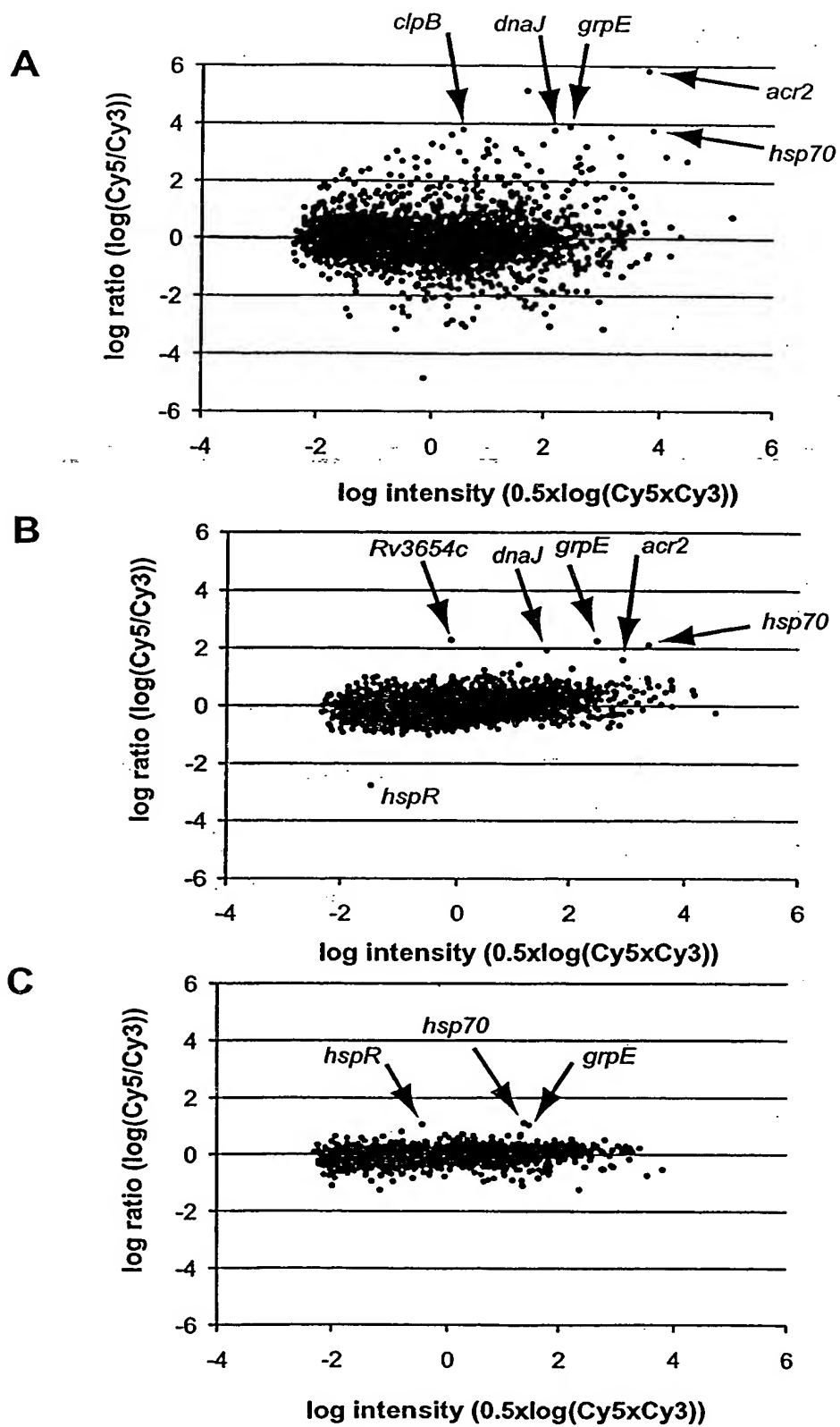


Figure 10

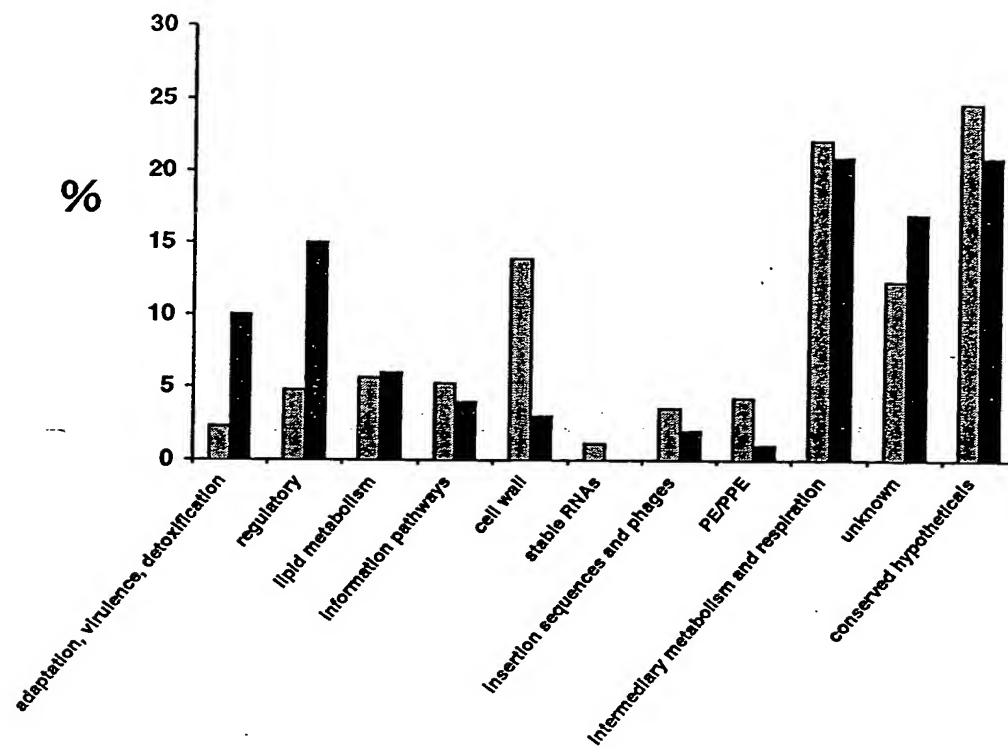


Figure 11

A

<i>acr2</i>	(Rv0251c)	AGGTGGAACTTAAGCGTGGTCCACTCAGGTCTTGTT	-61
<i>hsp70</i>	(Rv0350)	CTCAGTAAGTTGAGTGCATCAGGCGAGGCTCTCAATT	-89
<i>hsp70</i>	(Rv0350)	GAGGCAAGCCTGAGCGGGGTGCCTCATCAAACTGCA	-35
<i>clpB</i>	(Rv0384c)	TGGGTAAAAATCAGCGGAACAGACTCAACATTGACGG	-50
<i>M.tb</i> consensus HAIR		A--TTGAGCG-----GACTCA-C-T-G	

B

<i>groEL2</i>	(Rv0440)	GAATAACGTTGCACTCGCGACCGTCACTGCTAGGTCTG	-188
<i>groEL2</i>	(Rv0440)	CGGGGCTTCTTCACTCGGCATAGCGAGTGGTAAGAAT	-153
<i>Rv0991c</i>		TAGCGGTTCTACCACTTGAGACGGTAGACTGGTAACGCC	-23
<i>groES</i>	(Rv3418c)	CTTGAGTGGTASCACCTCTCATGTATAGACTGCTAGATGG	-168
<i>M.tb</i> consensus CIRCE		T-CT-GCACTCG--A--G--GAGTGCTA	

Figure 12

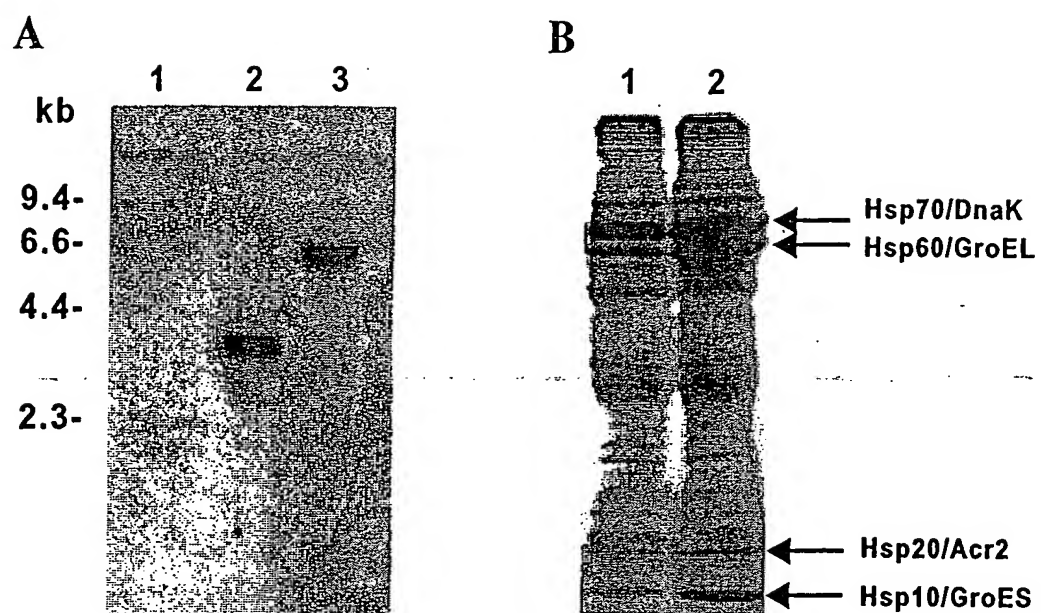


Figure 13

Table 1.

Gene designation	Gene name	P value	Fold change in Δ hspR compared to wild-type	Fold change in the complemented strain, Δ hspR pSMT168, compared to wild-type	Regulatory sequence	Function
Rv0004		4.93E-03	2.77	1.19		CH
Rv0108c		4.12E-04	2.09	1.16		unknown
Rv0249c		1.06E-03	2.00	1.10	HAIR	membrane protein
Rv0250c		2.93E-05	2.26	1.15	HAIR	CH
Rv0251c	<i>acr2</i>	2.14E-07	2.54	1.11	HAIR	chaperone
Rv0268c		5.46E-03	1.96	1.23		unknown
Rv0291		7.37E-03	2.93	0.84		membrane protease
Rv0350	<i>dnaK</i>	2.49E-25	4.50	2.23	HAIR	chaperone
Rv0351	<i>grpE</i>	1.58E-28	4.99	2.12	HAIR	chaperone
Rv0352	<i>dnaJ</i>	1.40E-19	4.01	1.60	HAIR	chaperone
Rv0455c		3.89E-06	2.32	1.25		unknown
Rv0469	<i>umaA1</i>	2.15E-04	2.06	0.98		mycolic acid synthesis
Rv0516c		1.19E-04	2.16	1.15		unknown
Rv0569		1.06E-03	1.98	0.62		CH
Rv0635		1.50E-04	2.13	1.05		CH
Rv0643c	<i>mmaA3</i>	5.09E-04	2.04	0.88		mycolic acid synthesis
Rv0655		9.26E-03	1.82	1.11		abc transporter
Rv0701	<i>rplC</i>	7.34E-03	1.95	1.27		ribosomal protein
Rv0714	<i>rplN</i>	2.55E-03	2.00	1.20		ribosomal protein
Rv0715	<i>rplX</i>	2.01E-03	2.03	1.10		ribosomal protein
Rv0909		1.90E-03	1.98	-		CH
Rv1078	<i>pra</i>	4.08E-04	2.00	1.09		CH
Rv1172c		2.27E-03	1.99	1.42		PE protein family
Rv1174c		2.90E-03	1.93	1.10		unknown
Rv1298	<i>rpmE</i>	4.64E-04	2.11	1.30		ribosomal protein
Rv1738		6.42E-07	2.46	0.89		CH
Rv1872c	<i>lldD2</i>	1.02E-03	1.93	1.14		oxidoreductase
Rv2005c		5.43E-04	2.03	0.83		universal stress protein
Rv2007c	<i>fdxA</i>	2.04E-14	3.17	0.86		ferredoxin
Rv2069	<i>sigC</i>	7.07E-03	1.94	1.16		sigma factor
Rv2094c	<i>tatA</i>	2.34E-04	2.17	1.15		protein translocase
Rv2137c		9.95E-04	1.97	1.09		CH
Rv2185c		7.65E-04	2.07	1.02		CH
Rv2193	<i>ctaE</i>	2.05E-04	2.02	1.22		cytochrome-c-oxidase
Rv2288		6.64E-04	2.09	1.08		unknown
Rv2289	<i>cdh</i>	6.34E-03	1.87	1.03		cdp-diglyceride hydrolase
Rv2764c	<i>thyA</i>	8.29E-03	1.83	1.10		thymidylate synthase
Rv2816c		3.93E-10	2.84	1.59		CH
Rv2876		1.89E-03	1.90	1.20		unknown
Rv2960c		1.03E-03	2.04	1.04		unknown
Rv3101c	<i>fisX</i>	3.61E-03	1.92	1.04		cell division protein
Rv3128c		2.12E-03	1.96	1.02		CH
Rv3407		1.44E-04	2.10	1.15		CH
Rv3418c	<i>groES</i>	4.45E-03	1.92	1.06		chaperone
Rv3508		9.24E-03	1.90	-		PE-PGRS protein family
Rv3654c		1.34E-27	5.08	1.53		unknown
Rv3679		2.57E-03	1.96	1.12		anion transporter
Rv3680		5.91E-03	1.85	1.45		anion transporter
Rv3841	<i>bfrB</i>	7.64E-04	1.98	1.02		bacterioferritin

Figure 14

Table 2

Gene designation	Gene name	P value	Fold upregulated compared to wild-type	Regulatory sequence	Function
Rv0009	<i>ppiA</i>	8.63E-04	1.80		peptidyl-prolyl cis-trans isomerase
Rv0250c		9.78E-08	1.87	HAIR	CH
Rv0251c	<i>acr2</i>	8.24E-05	1.88	HAIR	chaperone
Rv0350	<i>dnaK/hsp70</i>	2.32E-27	5.55	HAIR	chaperone
Rv0351	<i>grpE</i>	1.52E-31	5.77	HAIR	chaperone
Rv0352	<i>dnaJ</i>	1.71E-16	2.71	HAIR	chaperone
Rv0440	<i>groEL2</i>	5.32E-22	4.60	CIRCE	chaperone
Rv0455c		2.15E-06	1.82		unknown
Rv0464c		5.29E-03	1.60		CH
Rv0469	<i>umaA1</i>	6.51E-06	1.81		mycolic acid synthesis
Rv0705	<i>rpsS</i>	2.95E-07	1.93		ribosomal protein
Rv0709	<i>rpmC</i>	3.19E-03	1.65		ribosomal protein
Rv0979c		1.74E-04	1.75		unknown
Rv0991c		3.58E-08	1.95	CIRCE	CH
Rv1182	<i>papA3</i>	7.35E-03	1.73		pks-associated protein
Rv1185c	<i>fadD21</i>	7.82E-07	1.83		acyl-coA synthase
Rv1233c		3.08E-03	1.68		CH
Rv1388	<i>mJHF</i>	4.73E-05	1.75		integration host factor
Rv1476		1.95E-03	1.60		membrane protein
Rv1642	<i>rpmL</i>	1.19E-05	1.83		ribosomal protein
Rv1721c		4.93E-03	3.10		CH
Rv1791		3.52E-03	1.60		PE protein family
Rv1860	<i>modD/vapA</i>	1.11E-03	1.65		fibronectin-binding protein
Rv1980c	<i>mpt64</i>	1.89E-10	2.49		secreted unknown
Rv2069	<i>sigC</i>	3.85E-03	1.62		sigma factor
Rv2081c		5.05E-03	1.60		unknown
Rv2094c	<i>latA</i>	1.16E-05	1.77		protein translocase
Rv2185c		2.09E-05	1.78		CH
Rv2271		1.55E-08	1.95		unknown
Rv2302		2.02E-05	1.83		CH
Rv2882c	<i>frr</i>	2.49E-04	1.74		ribosome recycling factor
Rv2928	<i>tesA</i>	1.14E-05	1.87		thioesterase
Rv2931	<i>ppsA</i>	7.16E-04	1.67		polyketide synthase
Rv2941	<i>fadD28</i>	1.44E-06	1.86		acyl-CoA synthetase
Rv2949c		1.70E-04	1.67		unknown
Rv3224		2.82E-04	1.67		oxidoreductase
Rv3281		2.99E-03	1.60		CH
Rv3390	<i>lpqD</i>	4.14E-03	1.63		secreted lipoprotein
Rv3417c	<i>groEL1</i>	1.19E-10	2.41	CIRCE	chaperone
Rv3418c	<i>groES</i>	8.22E-59	9.60	CIRCE	chaperone
Rv3528c		4.16E-04	1.79		unknown
Rv3615c		3.90E-03	1.65		CH
Rv3654c		5.17E-40	3.97		unknown
Rv3750c		1.25E-03	1.63		excisionase
Rv3786c		4.01E-04	2.74		unknown
Rv3846	<i>sodA</i>	5.24E-08	2.14		superoxide dismutase
Rv3852	<i>hns</i>	1.11E-04	1.69		histone-like protein
Rv3874		1.39E-04	1.82		CH
Rv3891c		7.12E-04	1.66		unknown

Figure 15